

COMPARATIVE ANALYSIS OF PROTEINS FROM CYTOPLASMATIC ORGANELLES OF POLLEN FROM CERTAIN COTTON SPECIES

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The protein composition of cytoplasmatic organelles within species, phylogenetic group, and genus was investigated using electrophoretic separation of protein fractions.

Key words: cotton, proteins of cytoplasmatic organelles.

The protein composition of cotton pollen has been reported [1]. A comparative analysis of the proteins from cytoplasmatic organelles has been performed. Representatives of the genomic group A *Gossipium herbaceum* and *G. arboreum* contain more similar protein fractions than representatives of genomic group AD. Proteins common to *G. herbaceum* and *G. arboreum* have R_f values for the salt-soluble proteins of 0.06, 0.07, 0.08, 0.13, 0.14, 0.24, and 0.25; those specific to *G. herbaceum*, 0.18 and 0.19; to *G. arboreum*, 0.21. Therefore, the phylogenetic link between species of one phylogenetic group are discernable here. There is also a similarity in the quantitative sense: nine bands are isolated from *G. herbaceum*; eight, from *G. arboreum*. Two principal protein fractions are isolated from *G. herbaceum* and *G. arboreum*. These have R_f values 0.24 and 0.25 for *G. herbaceum* and 0.08 and 0.21 for *G. arboreum* (Table 1). The spectra of R_f values for *G. herbaceum* and *G. arboreum* are similar and vary in the range 0.06-0.25.

G. thurberi also typically contains proteins with molecular weights that exceed 60 kD (Table 1). The proteins of *G. thurberi* include more dark-orange bands than those of other species. These are proteins with R_f values 0.24, 0.26, 0.27, and 0.31. The R_f values of proteins from cytoplasmic organelles vary in the range 0.04-0.5.

The R_f values of salt-soluble proteins of *Hibiscus* sp. lie in the range 0.07-0.33. One dark band is isolated with R_f 0.18. Primarily proteins of molecular weight >60 kD are found in *Hibiscus* sp.

A characteristic feature within the genus is the presence of high-molecular weight protein fractions. Many similar fractions are obtained from representatives of genomic group A *G. arboreum* and *G. herbaceum*.

Thus, a study of the protein spectrum of certain *Gossipium* and *Hibiscus* sp. revealed the presence of similar protein fractions within the phylogenetic groups and to a lesser extent within species. This is consistent with the correct assignment of species to one group or another, i.e., the geographic criterion for a phylogenetic link is supported by the biochemical properties. The materials were graciously supplied by the Institute of Plant Genetics and Selection of the Academy of Sciences of the Republic of Uzbekistan.

EXPERIMENTAL

Cotton varieties 108-F *G. hirsutum* L. and C-6037 *G. barbadense* were mainly studied. In separate experiments, we also used pollen of cotton species differing in evolutionary development, chromosome number, and several economic indicators: *G. hirsutum* L. (AD₁) and *G. barbadense* (AD₂), *G. herbaceum* L. (A₁), *G. arboreum* (A₂), *G. thurberi* Tod (D₁), and *Hibiscus* sp.

Fully opened flowers were collected at a certain time of day from plants grown under field conditions. Pollen was shaken onto foil and collected in containers (for protein analysis, in hermetically sealed weighing bottles).

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TABLE 1. R_f Values of Protein Fractions from Cytoplasmatic Organelles of Pollen from Certain Cotton Species

Species	R_f	Total protein fractions
<i>G. hirsutum</i> (AD)	0.09, 0.16, 0.22, 0.33, 0.35	5
<i>G. barbadense</i> (AD)	0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.19, 0.22, 0.24, 0.3, 0.37	11
<i>G. herbaceum</i> (A)	0.06, 0.07, 0.08, 0.13, 0.14, 0.18, 0.19, 0.24, 0.25	9
<i>G. arboreim</i> (A)	0.06, 0.07, 0.08, 0.013, 0.14, 0.21, 0.24, 0.25	8
<i>G. thurberi</i> (D)	0.04, 0.05, 0.06, 0.21, 0.24, 0.26, 0.27, 0.28, 0.31, 0.5	10
<i>Hibiscus sp.</i>	0.07, 0.08, 0.12, 0.15, 0.18, 0.19, 0.21, 0.28, 0.33	10

Fractions containing generative cells and those containing proteins from cytoplasmatic organelles were treated with extraction buffer (1.5 mL, 0.7 M saccharose, 0.5 M Tris, 30 mM HCl, 50 mM EDTA, 0.1 M KCl, and 2% mercaptoethanol) (6). Each sample was incubated for 10 min at 4°C and mixed with an equal volume of water-soluble phenol. Then, TRITON X-100 in urea buffer was added to the solutions to a concentration of 0.05%. After a 2-hour incubation, the solutions were centrifuged at 14000g for 20 min. The supernatant was used for the determination of protein content.

Electrophoresis of the total salt-soluble proteins was performed on a Laemmli MK instrument using reagent-grade materials (Reanal) in 12% polyacrylamide gel [2].

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